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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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QPA AND HIV-INTEGRASE APTAMER IN THE PRESENCE OF LEAD IONS

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Abstract.

Objective: The aim of this research was to implement Quadruplex Priming Amplification (QPA) and to perform a spectroscopic study of an HIV-integrase aptamer in the presence of lead ions.

Methods: Quadruplex Priming Amplification (QPA) is a simple amplification assay in which isothermal amplification is performed using only a DNA polymerase, and detection is conducted by the intrinsic fluorescence of the primers. QPA employs specific G-rich sequences (G3T) as primers that, upon polymerase elongation at defined temperatures, spontaneously dissociate from the primer-binding sites (PBS) and fold into a monomolecular quadruplex. The G3T sequence is based on a DNA aptamer designed against HIV-1 integrase. Fluorescent nucleotide analogs, such as 3-methyl isoxanthopterin (3MI), when incorporated into these primers, emit light upon quadruplex formation and permit simple, specific, and sensitive quantification without the need for attached probe molecules.

Results: Previously, QPA assays were developed using truncated targets and potassium cations. Here, we designed QPA assays with a truncated target and lead cations within a temperature range of 50–60 °C. Lead cations resulted in significantly faster amplification compared with potassium cations. In addition, we performed a spectrophotometric study of the G3T sequence in the presence of lead cations. Lead cations exhibited highly stable quadruplex formation.

Conclusion: The target molecule can be extended with a complementary sequence derived from a pathogen, enabling pathogen detection. QPA can be applied as a simple and inexpensive diagnostic method for point-of-care (POC) applications, as well as for the development of faster amplification strategies, which will make this method more suitable for molecular diagnostics.

Although we use low concentrations, given the toxicity of lead, suitable disposal methods are imperative.

Key words. DNA amplification, quadruplex, the point of care, lead cations, fluorescence.

Introduction.

Current systems for clinical diagnostic applications are mainly PCR-based, can typically only be used in hospital settings, and are still relatively complex and expensive. Integrating sample preparation with nucleic acid amplification and detection in a cost-effective, robust, and user-friendly format therefore remains challenging [1].

The free energy of the d(GGGTGGGTGGGTGGG) (G3T) quadruplex can be used to drive isothermal amplification of DNA, referred to as Quadruplex Priming Amplification (QPA) [2]. QPA allows isothermal amplification of nucleic acids with improved yield and simplified detection. This assay is based on the G3T DNA quadruplex, which, in the presence of specific cations, possesses unusually high thermal stability. The key point of QPA is that the G3T sequence is capable of forming quadruplexes with significantly more favorable thermodynamics than the corresponding DNA duplexes. QPA employs truncated G3T sequences as primers, which, upon polymerase elongation, self-dissociate from the binding site and allow the next round of priming without thermal unfolding of amplicons. In addition, G3T primers with an incorporated 3-methyl isoxanthopterin (3MI) in the fourth position demonstrated a 50-fold increase in fluorescence upon quadruplex formation, which allows simple, specific, and effective quantification without the use of extra probe molecules [3,4]. 3MI, with excitation and emission wavelengths of 348 nm and 431 nm, respectively, is a fluorescent analog of guanine, although 3MI does not base pair with natural nucleic-acid bases and has a high quantum yield (0.88) [5]. Upon incorporation even into unstructured single-stranded DNA, its fluorescence is significantly quenched. This limits the widespread use of 3MI in nucleic acid reactions; as such, it has been mainly used to study nucleic acid loop structures.

As we have mentioned, this assay is based on the G3T DNA quadruplex. Quadruplexes play important roles in many biological processes [6-9]. The G3T sequence is based on a DNA aptamer that was designed against HIV-1 integrase [10]. Monomolecular quadruplexes are formed by stacks of G-quartets connected to each other by single-stranded loops. G-quartets are formed by four guanine residues associated in a square planar configuration, in which each guanine interacts with its two neighbors through hydrogen bonds (eight per quartet). The formation of G-quartets requires the presence of cations (such as K^+), which bind specifically to guanine O6 carbonyl groups between the planes of the G-quartets [11]. Due to cation coordination in the center of G-quartets and stacking interactions, monomolecular quadruplexes are remarkably stable and fold readily [12]. The truncated version of G3T is not able to fold into a quadruplex and instead perfectly hybridizes with the template, which represents a complementary sequence to G3T. A polymerase then attaches missing guanines and, as a result, G3T spontaneously dissociates from its template, folds into the quadruplex, and emits light. As a result, QPA is able to amplify DNA isothermally, which is critical for DNA-based point-of-care diagnostics.

Lead(II) chloride ($PbCl_2$) is an inorganic compound that is a white solid under ambient conditions. It is poorly soluble in water. Lead(II) chloride is one of the most important lead-based reagents [9,13]. It also occurs naturally in the form of the mineral cotunnite. In solid $PbCl_2$, each lead ion is coordinated by nine chloride ions in a tricapped triangular prism formation—six located at the vertices of a triangular prism and three positioned beyond the centers of each rectangular prism face. The nine chloride ions are not equidistant from the central lead atom;

seven lies at distances of 280–309 pm, and two at 370 pm [14]. $PbCl_2$ forms white orthorhombic needles.

Experimental Procedures.

Enzymes and DNA Substrates:

DNA polymerase: Vent (exo-) was purchased from New England BioLabs. Vent (exo-) DNA polymerase has been genetically engineered to eliminate the 3′→5′ proofreading exonuclease activity associated with Vent DNA Polymerase [15]. This is the preferred form for high-yield primer extension reactions. The fidelity of polymerization by this form is reduced to a level of about two-fold higher than that of Taq DNA Polymerase [16,17].

DNA oligonucleotides were obtained from Integrated DNA Technologies and Fidelity Systems. All measurements were performed in a buffer solution consisting of 10 mM Tris-HCl, pH 8.7.

UV-Visible Spectroscopy:

Temperature-dependent UV-Visible spectroscopy is a convenient tool to study secondary structure, thermal stability, and to estimate van't Hoff thermodynamics of DNA or RNA molecules. UV-Visible spectroscopy is traditionally used to monitor thermal unfolding experiments of nucleic acids. Specifically, the unfolding of DNA duplexes is accompanied by an increase in absorbance at 260 nm, and accurate determination of the thermodynamic parameters for quadruplexes is possible by monitoring the long-wavelength region of the UV spectrum (~295 nm) [18-20].

UV unfolding experiments allowed estimation of the melting temperature of the quadruplex. Melting temperatures were determined by recording UV absorption at ~295 nm as a function of temperature using a Varian UV–visible spectrophotometer (Cary 100 Bio) using ~4 µM G3T oligonucleotide solutions in 1 cm path-length cells. In a typical experiment, oligonucleotide samples were mixed and diluted into the desired buffers in quartz cuvettes. After annealing by heating to 97 °C, the temperature was ramped to the desired starting temperature, different concentrations of $PbCl_2$ were added, and the melting experiments were performed at a heating rate of 1 °C/min. The UV melting curves allowed an estimate of the melting temperature, Tm, the midpoint temperature of the unfolding process [21].

Fluorescence Spectroscopy:

Fluorescence spectroscopy is one of the most sensitive analytical tools. Fluorescence has traditionally been used to monitor the activity of various enzymes, to quantify DNA amplification, and to study molecular interactions and dynamics. In our assays, we use fluorescent nucleotide analogs (e.g., 3MI), which are completely quenched in single-stranded or double-stranded DNA and emit light upon quadruplex formation. Fluorescence measurements were performed using an ESEQuant Tube Scanner (Qiagen). QPA reactions were carried out in a reaction mixture (volume: 0.1 mL) containing 300 nM primer, 30 nM template, 800 µM dGTP, buffer (50 µM $PbCl_2$, 50 mM CsCl, 2 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.7), and 0.06 or 0.07 unit/µL Vent (exo-) polymerase. The reactions were carried out directly in 0.2 mL PCR tubes. In a typical experiment, buffer, DNA polymerase, and dGTP were added, and the fluorometer was equilibrated at the reaction temperature, followed by real-time fluorescence monitoring.

Results and Discussion.

G3T quadruplex (HIV-integrase aptamer) by lead cations:

As we mentioned before, cations are necessary for quadruplex formation; however, not all cations are able to induce quadruplex formation, or they may form quadruplexes with lower stability. For example, potassium cations can form stable quadruplexes, whereas cesium cations cannot. This is because K^+ ions, with an ionic radius of 133 pm, have an optimal size to fit into the inner core of G-quartets, whereas Cs^+ ions, with an ionic radius of 169 pm, are too large [5,22]. Therefore, Cs^+ ions allow the ionic strength to be maintained without affecting or inducing the formation of additional quadruplex species.

We performed UV melting experiments of 4 µM G3T quadruplex at different concentrations of Pb^{2+} cations. At low concentrations of Pb^{2+} (8 µM), the experiments revealed reversible two-state transitions. At this concentration, both unfolding (solid lines) and refolding (dashed lines) curves were sigmoidal and superimposable, which is characteristic of fully reversible two-state transitions (Figure 1). However, at higher concentrations of Pb^{2+} cations, the unfolding curves became more complex. In the presence of 50 and 100 µM Pb^{2+}, G3T exhibited unusually large and reproducible hysteresis. Similar hysteretic behavior was previously observed for two conjugated G3T monomers, $(G3T)_2$, and was attributed to a highly specific two-state transition in which folding and unfolding of the first G3T monomer is rate-limiting for both annealing and melting processes. Thus, the hysteresis observed in the presence of Pb^{2+} ions can be attributed to dimerization of G3T quadruplex monomers [23].

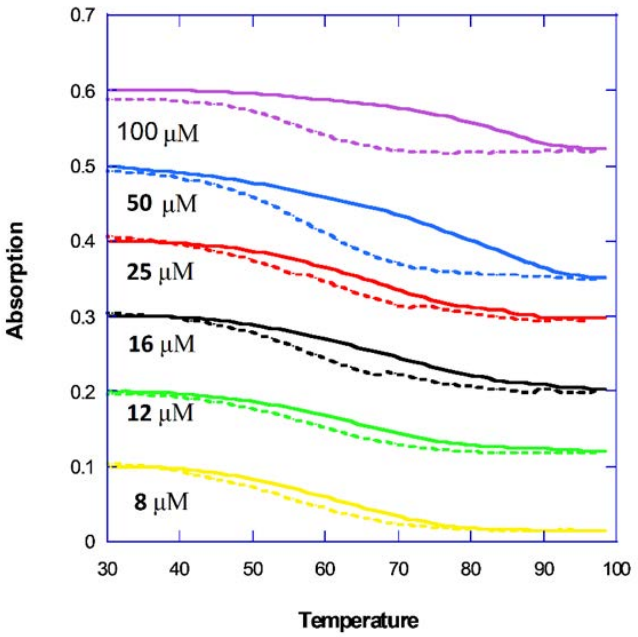

Figure 1. *Melting of G3T quadruplex in the presence of different concentrations of Pb^{2+} cations.*

Primer/Template Complex for QPA:

For this task, we designed specific primer and template molecules. The primer 5′-GGG(3MI)GGGCGGGTGG-3′ represents a modified G3T sequence containing an incorporated 3MI residue at the fourth position and lacking one guanine at the 3′ end. As a result, the estimated melting temperature of the substrate was 51.9 °C (Table 2).

QPA by lead cations:

For comparison, we used 25 mM K^+, which is the optimal concentration for QPA reactions with potassium cations [3]. In this experiment, the highest QPA rate was observed in the presence of Pb^{2+} cations. QPA performed with 50 µM Pb^{2+} resulted in approximately five-fold faster amplification compared with 25 mM K^+ at 53 °C (Figure 2).

The results show (Figure 2) that once the amplification reaches the plateau phase and the fluorescence signal reaches its maximum (corresponding to the maximum number of quadruplexes), no subsequent decrease in signal is observed. This indicates that possible dimerization of the quadruplexes does not interfere with the fluorescent signal. In addition, in the main experiments (Figure 3) we used a lead concentration of 10 µM/L, at which there is almost no hysteresis (Figure 1) and therefore dimerization may be less.

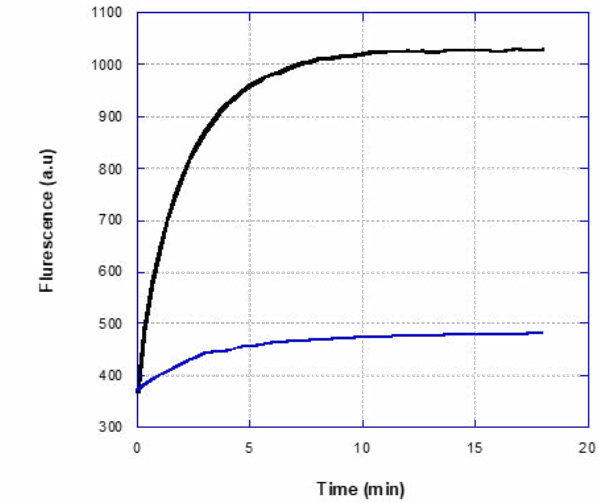

Figure 2. *QPA reactions using 50 µM Pb^{2+} (black) and 25 mM K^+ (blue) at 53 °C in 10 mM Tris-HCl buffer (pH 8.7) containing 50 mM CsCl and 2 mM $MgCl_2$, employing Vent (exo-) DNA polymerase.*

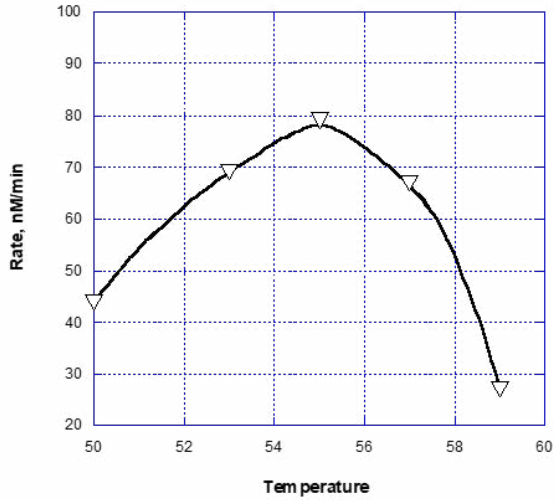

Figure 3. *Temperature dependence of QPA rates using the Substrate listed in Table 1. Primer/template concentrations: 300/30 nM, employing 0.06 unit/µl Vent (exo-), 800 µM of dGTP in 10 µM $PbCl_2$, 50 mM CsCl, 2 mM $MgCl_2$, Tris-HCl 10 Mm, pH 8.7.*

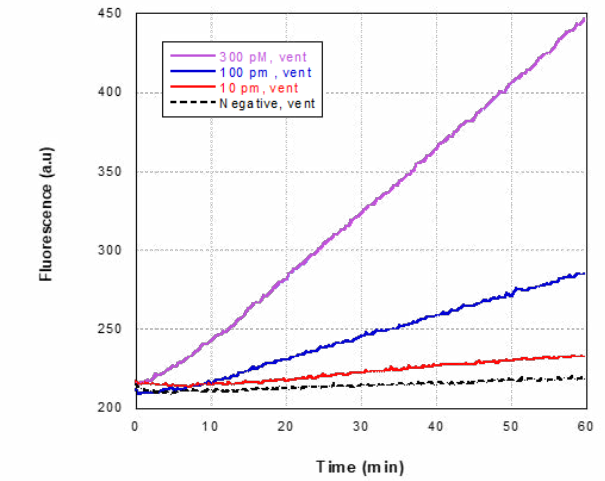

Figure 4. *QPA reactions at low concentrations of template molecule using Substrate, Primer 500 nm, template: 300 pM (purple), 100 pM (blue), 10 pM (red) and negative control (black). Employing 0.07 unit/ µl vent (exo-), 800 µM of dGTP in 10 µM $PbCl_2$, 50 mM CsCl, 2mM $MgCl_2$, Tris-HCl 10 Mm, pH 8.7 at 57 °C, volume: 0.1 mL.*

We also performed QPA measurements for a negative control in the presence of Pb^{2+} cations, which contained all reaction components except the template. As expected, no amplification was observed, because DNA polymerase cannot incorporate the missing guanine in the absence of duplex formation, and the primer alone is unable to fold into a quadruplex.

In this study, we implemented Quadruplex Priming Amplification in the presence of lead ions. QPA reactions were carried out within a temperature range of 50–60 °C at 2 °C intervals using the substrate. QPA was monitored by fluorescence of 3MI incorporated at the fourth position of the primer. The reaction mixture contained 300 nM primer and 30 nM template. Average QPA rates were determined. The substrate exhibited strong activity across the 50–60 °C range, with an optimal rate observed at 55 °C, reaching approximately 80 nM/min (Figure 3). The rate is measured at the beginning of the graph (amplification) by the slopes.

To determine the efficiency of QPA with lead ions, we performed measurements at low concentrations of the template molecule. The results show that linear QPA mediated by lead ions enables the detection of a 10 pM template molecule in less than one hour (Figure 4).

In the study we use a very small amount of lead ion, 0.2 micrograms is sufficient for full amplification (Fig.4). For comparison: Although no safe level for lead exposure has been identified a blood lead level of < 2 micrograms per deciliter (20 µg /L) is considered normal [24]. An interim reference level (IRL) for lead based on the Centers for Disease Control and Prevention's (CDC) blood reference level of 3.5 micrograms

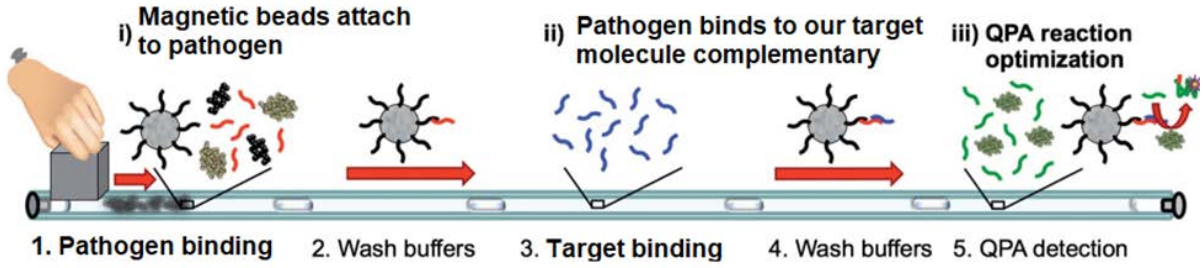

Figure 5. *Magnetic bead-based approach for Point of care diagnostics using the QPA reaction (red-pathogen, blue-target molecule, green-QPA primer) [26].*

of lead per deciliter of whole blood (µg /dL). The amount of lead intake from food that would be required to reach the CDC's blood reference level are 2.2 micrograms (µg) per day for children and 8.8 µg per day for females of childbearing age [25]. The mass of lead used in our experiment is ten times smaller than this amount. Although experimental waste must be appropriately managed in accordance with local regulations.

One of the methods for pathogen detection is a magnetic bead–based approach for point-of-care diagnostics. In this method, the pathogen attaches to magnetic beads and can be transported through different chambers using a magnet. During this movement, the pathogen (for example, DNA of Mycobacterium tuberculosis or RNA of COVID-19) can hybridize with a complementary target molecule. For RNA targets, either complementary DNA/RNA hybrid molecules—can be used. In the third step (Fig. 3), the target-bound pathogen is transported to the amplification/detection chamber. Subsequently, the QPA reaction and the resulting fluorescence signal indicate the presence of the pathogen in the solution (Figure 5). In addition, all chambers are isolated from both patients or medical personnel and will not have direct contact, including with lead ions, which will be placed in the fifth chamber along with other QPA reagents from the beginning.

Because a POC applications, once used, contains a patient's blood sample or another biological specimen, it may potentially contain pathogens (this applies regardless of whether the test produces a positive or negative result, since the test may be designed to detect a specific pathogen while other pathogens may still be present in the sample) therefore, final incineration (burning) of applications is required. (Moreover, as already mentioned with regard to lead, blood itself contains a higher concentration of lead (20 µM/L) than will be used in the application (10 µM/L)).

Conclusion.

In the present work, we designed linear QPA assays operating within a temperature range of 50–60 °C in the presence of lead ions. The substrate (Table 2) demonstrated strong activity throughout this interval, enabling detection of target molecules at concentrations as low as 10 pM. The results show that QPA performed with lead cations exhibits approximately five-fold faster amplification compared with potassium cations. UV/Vis spectroscopic experiments demonstrated that G3T forms a highly stable quadruplex in the presence of Pb^{2+} cations, with a melting temperature of approximately 85 °C for 4 µM G3T and 100 µM Pb^{2+}, compared with ~57 °C in the presence of potassium cations. These findings indicate that QPA is a promising approach for simple and inexpensive point-of-care (POC) diagnostics, and that the use of lead cations significantly enhances amplification speed, making this method more suitable for molecular diagnostic applications.

Table 1. *Melting temperature of G3T quadruplex in the presence of different concentrations of Pb^{2+} cations.*

Concentration of Pb^{2+}, µM	*Tm* (°C)
8	61
12	64
20	67
25	68
50	82
100	85

Tm values (within ±1°C) were derived from UV melting curves (Figure 1) at a concentration of 4 µM G3T; Buffer: 10 mM Tris–HCl, pH 8.7.

Table 2. *Primer/Template Complex.*

Primer/Template Complexes	Name	*Tm* [a] (°C)
5'-GGG(**3Mi**)GGGCGGGTGG 3'- CCCGCCCACCC	Substrate	51.9

[a]*Tm values were estimated from nearest-neighbor analysis. Concentration of Primer/Template Complexes 300 nM, 50 mM monovalent cations, 2mM $MgCl_2$.*

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Compliance with ethical standards.

Conflict of interest.

The authors declare that they have no conflict of interest.

Ethical approval.

This article does not contain any studies with human participants or animals performed by any of the authors.

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რეზიუმე

მიზნები:კვლევისმიზანიაკვადრუპლექს-პრაიმერული ამპლიფიკაციის (QPA) და HIV ინტეგრაზას აპტამერის სპექტროსკოპიული კვლევის განხორციელება ტყვიის იონების გამოყენებით.

მეთოდები:კვადრუპლექს-პრაიმერულიამპლიფიკაცია (QPA) არის ძალიან მარტივი ამპლიფიკაციის მეთოდი, რომლის დროსაც იზოთერმული ამპლიფიკაცია ხორციელდება მხოლოდ დნმ პოლიმერაზას გამოყენებით და დეტექცია ხორციელდება პრაიმერების შინაგანი ფლუორესცენციით. QPA იყენებს სპეციფიკურ G-მდიდარ თანმიმდევრობებს (G3T), როგორც პრაიმერებს,რომლებიცპოლიმერაზას მიერ სპეციფიკურ ტემპერატურაზე ელონგაციისას, სპონტანურად დისოცირდებიან პრაიმერის შეკავშირების ადგილებიდან (PBS) და იკეცებიან მონომოლეკულურ კვადრუპლექსად. G3T თანმიმდევრობა დაფუძნებულია დნმ აპტამერზე, რომელიც შექმნილია აივ-1 ინტეგრაზას წინააღმდეგ. ფლუორესცენციული ნუკლეოტიდური ანალოგები, როგორიცაა 3-მეთილ იზოქსანთოპტერინი (3MI), ამ პრაიმერებში ინტეგრირებისას ასხივებენ სინათლეს კვადრუპლექსის წარმოქმნისას და იძლევიან მარტივ, სპეციფიკურ და მგრძნობიარე რაოდენობრივ განსაზღვრას ზონდის მოლეკულების მიმაგრების გარეშე.

შედეგები: ადრე შემუშავებული იყო QPA სამიზნე მოლეკულითა და კალიუმის კათიონებით. აქ ჩვენ შევიმუშავეთ QPA მეთოდი ტყვიის კათიონებით 50-60 °C ტემპერატურულ ინტერვალში. ტყვიის კათიონები ავლენენ ბევრად სწრაფ ამპლიფიკაციას, ვიდრე კალიუმის კათიონები. სამიზნე მოლეკულას შეიძლება მიმაგრდეს პათოგენის კომპლემენტარული

თანმიმდევრობა, რაც გვაძლევს პათოგენის აღმოჩენის შესაძლებლობას. გარადა ამისა მოვახდინეთ G3T თანმიმდევრობის სპექტროფოტომეტრიული კვლევა. ტყვიის კათიონმა აჩვენა ძალიან სტაბილური კვადრუპლექსის ფორმირება.

დასკვნა: QPA შეიძლება გამოყენებულ იქნას როგორც მარტივი და იაფი დიაგნოსტიკის (POC) განხორციელების მეთოდი, ასევე უფრო სწრაფი ამპლიფიკაციის შემუშავება ამ მეთოდს მოსახერხებელს გახდის მოლეკულურ დიაგნოსტიკაში გამოსაყენებლად.

Резюме

Цель: Цель исследования – внедрение квадруплексной праймирующей амплификации (QPA) и спектроскопическое исследование аптамера ВИЧ-интегразы в присутствии ионов свинца.

Методы: Квадруплексная праймирующая амплификация (QPA) – это очень простой метод амплификации, при котором изотермическая амплификация проводится с использованием только ДНК-полимеразы, а детектирование осуществляется по собственной флуоресценции праймеров. В QPA используются специфические G-богатые последовательности в качестве праймеров (G3T), которые при удлинении полимеразы при определенных температурах спонтанно диссоциируют от сайтов связывания праймеров (PBS) и сворачиваются в мономолекулярный квадруплекс. Последовательность G3T основана на ДНК-аптамере, разработанном против ВИЧ-1 интегразы. Флуоресцентные аналоги нуклеотидов, такие как 3-метилизоксантоптерин (3MI), при включении в эти праймеры излучают свет при образовании квадруплекса и позволяют проводить простое, специфичное и чувствительное количественное определение без присоединения молекул-зондов.

Результаты: Ранее были разработаны QPA-анализы с укороченными мишенями и катионами калия, данной работе мы разработали QPA-анализы с укороченной мишенью и катионами свинца в температурном интервале 50-60 °C. Катионы свинца демонстрируют более быструю амплификацию, чем катионы калия. Кроме того, мы провели спектрофотометрическое исследование последовательности G3T с катионами свинца. Катион свинца продемонстрировал очень стабильное образование квадруплекса.

Заключение: К целевой молекуле можно присоединить комплементарную последовательность патогена, что дает возможность обнаружения патогена. QPA может применяться в качестве метода для реализации простой и недорогой диагностики (в месте оказания медицинской помощи (POC)), а также для разработки более быстрой амплификации, что сделает этот метод удобным для использования в молекулярной диагностике.